ELSEVIER

Biochimica et Biophysica Acta 1311 (1996) 204–210

BBA Biochimica et Biophysica Acta

Inhibition of the expression of ornithine decarboxylase by some κ-opioidergic receptor ligands in difluoromethylornithine-resistant L1210 cells

Flavio Flamigni *, Claudio Stefanelli, Ivana Stanic, Claudio Muscari, Antonella Giaccari, Carmen Rossoni

Dipartimento di Biochimica 'G. Moruzzi', Università di Bologna, Via Irnerio 48, 40126 Bologna, Italy

Received 31 July 1995; revised 15 November 1995; accepted 11 January 1996

Abstract

In difluoromethylornithine resistant L1210 cells stimulated to growth from quiescence, the selective κ-opioidergic agonist *trans*-(±)-3,4-dichloro-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide (U-50488H) caused a dose dependent inhibition of the induction of ODC activity, with a half-maximal effect at about 1 μM. U-50488H also provoked reduction of ODC mRNA level and increase of ODC turnover, as well as inhibition of cell growth. U-69593, another κ-selective agonist, was only slightly effective. The action of U-50488H on ODC induction was not blocked by naloxone, β-chlornaltrexamine or by the κ-selective opioid antagonists Mr1452 and nor-binaltorphimine (nBNI). Actually Mr1452 and nBNI exerted some inhibitory effect. Furthermore, the separated enantiomers (+) and (−) of U-50488H were similarly effective. The (−)*cis*-(1*S*,2*R*)-U50488 stereoisomer, exhibiting low affinity for κ and high affinity for σ receptors and carbetapentane, another σ ligand, also inhibited ODC induction, although less effectively than U-50488H. None of several other opioid ligands tested had significant effects on ODC induction. In conclusion, the inhibition of ODC expression by U-50488H does not involve classical, enantiospecific opioid receptors; rather, these results suggest the involvement of a distinct site of action linked to inhibition of lymphoid cell proliferation.

Keywords: Ornithine decarboxylase; Gene expression; Opioid; U-50488H

1. Introduction

Numerous studies indicate that opioids play an important role in the regulation of neural cell proliferation and act as DNA synthesis modulators during brain development ([1–3] and references therein). Alteration of DNA synthesis by opioids is not restricted to neural cells as it occurs in lung and breast cancer cells, and in the immune system as well [4–7]. The proliferation capacity of peripheral blood lymphocytes is impaired in opiate addicts and various endogenous and synthetic opioids can affect DNA synthesis and other biochemical and immune responses of lymphoid cells [6–8]. These various, even opposite effects may be mediated by different kinds of opioid receptors like the μ, δ and κ types, which have been described in lymphoid cells [6–9]. However some effects do not appear to involve these classical opioid receptors since were not blocked by naloxone [6,7]. Naloxone insensitive binding sites for opioids have been described also in neural and other nonneural cells [10–13].

Ornithine decarboxylase (ODC), the first and key enzyme in polyamine biosynthesis, is essential for cell proliferation and is induced following growth stimuli [14–16]. ODC gene is now recognized as an early response gene required for entry into S phase of the cell cycle and, according to a recent paper [17], as a proto-oncogene. ODC expression can be finely regulated at transcriptional, translational and post-translational levels by numerous hormones, growth factors and cytokines [16]. However only a

Abbreviations: ODC, ornithine decarboxylase; DFMO, α-difluoromethylornithine; U-50488H, *trans*-(±)-3,4-dichloro-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide methanesulfonate; nBNI, nor-binaltorphimine; β-CNA, β-chlornaltrexamine; U-69593, (5α,7α,8β)-(+)-*N*-methyl-*N*-(7-[1-pirrolidinyl]-1-oxaspiro[4,5]deo-8-yl)-benzeneacetamide; DAMGO, [D-Ala^2,*N*-Me-Phe^4,Gly^5-ol]-enkephalin; Mr1452,(−)-*N*-(3-furylmethyl)-α-normetazocine methanesulfonate; PCR, polymerase chain reaction.

* Corresponding author. Fax: +39 51 351224.

PII S0167-4889(96)00009-2

few studies have been dedicated to the effects of opiates and opioid peptides on ODC regulation. In vivo administration of morphine, methadone, β-endorphin or acetyl-β-endorphin was found to increase or decrease ODC activity in some tissues, particularly of developing rats [18–21]. In these in vivo experiments some effects may have been indirect and mediated by the nervous or endocrine systems. In cultured neuroblastoma x glioma hybrid cells, micromolar concentration of morphine, levarphanol and etorphine inhibited the induction of ODC activity [22], whereas in hippocampal slices β-endorphin, acetyl-β-endorphin and ala-enkephalinamide potentiated the stimulation of ODC activity [23].

In order to ascertain if opioids may affect ODC expression in lymphoid cells, we have utilized a line of L1210 cells selected for resistance to the ODC inhibitor difluoromethylornithine (L1210-DFMO^r) [24]. Because of gene amplification, these cells can express ODC at very high levels, while maintaining the usual mechanisms of regulation of the enzyme.

2. Materials and methods

2.1. Materials

L1210-DFMO^r cells and antibodies against mouse ODC were a generous gift from Dr. L. Persson (University of Lund, Lund, Sweden). L-[1-^{14}C]Ornithine was purchased from ICN Biomedicals (Irvine, CA) and DL-[3,4-^{3}H]DFMO from DuPont-New England Nuclear (Boston, MA). DFMO was kindly provided by the Merrell Dow Research Institute (Strasbourg, France). *Trans*-(±)-3,4-dichloro-*N*-[2-(1-pyrolidinyl)cyclohexyl]benzeneacetamide methanesulfonate (U-50488H) and the separated trans (+) and (−) enantiomers of U-50488H (U-53444E and U-53445E) were generously given by the Upjohn Co. (Kalamazoo, MI). (−)-*cis*-(1*S*,2*R*)-U50488, nor-binaltor-phimine (nBNI), β-chlornaltrexamine (β-CNA), haloperidol, carbetapentane and metaphit methanesulfonate were from RBI (Natick, MA). Naloxone, (5α,7α,8β)-(+)-*N*-methyl-*N*-(7-[1-pirrolidinyl]-1-oxaspiro[4,5]dec-8-yl) benzeneacetamide (U-69593), methanesulfonic acid, tetrodotoxin, carbachol, atropine and [D-Ala^2, *N*-Me-Phe^4, Gly^5-ol] enkephalin (DAMGO) were purchased from Sigma Chemical Co. (St. Louis, MO). Dynorphin A (1–13) was from Peninsula Laboratories (Belmont, CA) and (−)-*N*-(3-Furylmethyl)α-normetazocine methanesulfonate (Mr1452) from Boheringer Ingelheim Pharmaceuticals, Inc. (Ridgefield, CT). Leu-enkephalin and met-enkephalin were supplied by Serva (Heidelberg, Germany) and human β-endorphin by Fluka (Buchs, Switzerland). Molecular biology products were purchased from Boheringer Mannheim (Mannheim, Germany), Pharmacia (Uppsala, Sweden), Perkin Elmer (Norwalk, CT) or Sigma. Oligonucleotide primers for reverse transcriptase/PCR were synthesized with a ABI 391 DNA synthesizer (Applied Biosystems, Cheshire, UK) and then purified by reverse phase HPLC. The PCRs were carried out in a Cetus DNA thermal cycler (Perkin Elmer). Capillary electrophoresis was performed in a P/ACE System 2100 (Beckman Instruments, Fullertone, CA).

2.2. Cell culture and treatment

Mouse L1210-DFMO^r cells were routinely grown in the presence of 20 mM DFMO in RPMI 1640 medium containing fetal-calf serum, antibiotics and β-mercaptoethanol, as described by Persson et al. [20]. For experiments, cells were shifted to, and maintained in, a DFMO-free medium for at least 15 days. Then, quiescent cells (cell density $\geq 2 \cdot 10^6$/ml) were seeded at $2 \cdot 10^5$/ml in fresh medium containing 10% serum and treated with opioids or other drugs as indicated. Wild-type L1210 cells were utilized under the same experimental conditions. Cell viability was checked by the trypan blue exclusion test. At the time indicated after seeding, cells were harvested and washed with phosphate-buffered saline.

2.3. Determination of ODC activity and immunoreactive protein content

Cell extracts were prepared and assayed for ODC activity and immunoreactive content as previously described [25]. ODC activity was determined in the presence of L-[1-^{14}C]ornithine by measuring the amount of CO_2 released [25,26], and is expressed as U/mg of protein, where 1 unit corresponds to 1 nmol CO_2/h incubation. U-50488H did not affect ODC activity significantly when added directly to the assay mixture at the concentration of 10 μM. The content of immunoreactive ODC protein was determined by radioimmunoassay by using [^{3}H]DFMO-labeled ODC, as described by Seely and Pegg [27]. Protein content was measured in cell extracts according to Bradford [28].

2.4. Detection of ODC mRNA

ODC mRNA was detected in L1210-DFMO^r cells by combined reverse transcriptase/PCR analysis. Total RNA was isolated from about 10^7 cells using the guanidinium thiocyanate method [29]. Any contaminating DNA was removed by DNase treatment essentially as described by Halmekyto et al. [30]. This treatment was necessary because mouse genome contains many pseudogenes for ODC [15]. cDNA synthesis and PCR were performed by the same enzyme, r*Tth* DNA polymerase (Perkin Elmer) according to manifacturers' instructions. The reaction mixture contained 0.25 μg of total RNA extracted from cells, 5 U of r*Tth* DNA polymerase, 0.2 mM each dNTP and 100 pmol of each primer in a final volume of 50 μl. The PCR primers for mature ODC mRNA were designed so as

to yield a PCR product of 224 nt. The 5′ primer (5′-TCATAGCTGAGCCAGGCAGATA-3′) was targeted to a sequence in exon 9 of mouse ODC mRNA and the 3′ primer (5′-CTTGGGTCTCTTCTGCAGC-3′) recognized a sequence at the junction of exons 10 and 11. The reverse transcriptase/PCR consisted of a reverse transcriptase phase (60°C for 30 min, 94°C for 2 min) followed by amplification (94°C for 45 s, 60°C for 75 s). Each sample was amplified at a growing number of cycles (in the range from 15 to 27 cycles). PCR products were either visualized by ethidium bromide fluorescence after separation on 1.5% agarose gel electrophoresis or quantitated by capillary electrophoresis coupled to laser-induced fluorescence. As for capillary electrophoresis, PCR products were directly separated at 35°C in a 27 cm total length × 0.1 mm I.D. deactivated fused-silica capillary (Beckman Instruments). Before separation, the capillary was equilibrated for 30 min with a running solution containing 89 mM Tris/boric acid, 2 mM EDTA (pH 8.5), 0.5% hydroxy-propyl-methyl-cellulose (4,000 cps) and 3 μl/ml of the fluorescent intercalator YOPRO (Molecular Probes, Eugene, OR). The samples were injected by pressure for 5 s and separated within 20 min at a constant voltage of 4 kV. Detection was performed by a sensitive laser-induced fluorescence detector equipped with an argon lamp. Values were corrected by comparison with a 525 bp internal standard (Bio Ventures). The high sensitivity of this technique allowed detection of samples not visualized on agarose gels. For the determination of the relative amounts of ODC mRNA, kinetic PCR analysis was performed according to Sugiyama et al. [31]: logarithms of values obtained by capillary electrophoresis were plotted against the number of PCR amplification cycles and extrapolation of the straight lines (correlation coefficient $r = 0.99$) to zero amplification cycle gave the amounts of ODC mRNA before the PCR amplification.

2.5. Statistical analysis

Significant differences between groups were assessed by Student's *t*-test for independent measurements or, where indicated, by paired *t*-tests or two-way ANOVA.

3. Results

3.1. Effects of U-50488H and other opioid agonists

We have previously shown that dilution of high density L1210-DFMO^r cells in fresh medium containing serum leads to induction of ODC activity and immunoreactive ODC protein, supported by an accumulation of ODC mRNA [32]. Several opioid agonists, natural or synthetic and with various receptor type specificity, were tested for the ability to modulate the induction of ODC activity. Table 1 shows that only the κ selective agonists U-50488H and U-69593 were able to inhibit the induction of the enzyme significantly. As shown in Fig. 1, both drugs exerted a dose dependent effect on ODC induction, but U-50488H was far more effective with a half-maximal effect at about 1 μM and an inhibition by about 90% at 10 μM. A significant effect was observed at a concentration of U-50488H as low as 0.2 μM ($P < 0.01$ vs. control). Since U-50488H is provided as a methanesulfonate salt, control experiments were performed with methanesulfonic acid alone (10 μM) and showed no significant effect on ODC induction. It should be noted that treatment with 10 μM U-50488H for 16 h, as employed in the previous experiments, did not affect cell viability; instead U-50488H (10 μM) inhibited cell growth markedly (Fig. 2). The time-course of the induction of ODC activity in the absence or in the presence of 10 μM U-50488H is shown in Fig. 3A. ODC induction was inhibited after 4 h of treatment ($P < 0.05$, significantly different from control) and remained low thereafter. U-50488H was effective even when administered 16 h after cell seeding to cells containing high levels of the enzyme (Fig. 3B): ODC activity was

Table 1
Effect of some opioid receptor agonists on the induction of ODC activity in L1210-DFMO^r cells

Opioid agonist	ODC activity (% of control)
morphine	97.4 ± 12.3
dynorphin A (1–13)	97.0 ± 8.9
β-endorphin	96.5 ± 1.4
Leu-enkephalin	102.9 ± 4.6
Met-enkephalin	100.0 ± 0.3
DAMGO	100.2 ± 14.2
U-50488H	10.9 ± 3.7 **
U-69593	83.5 ± 7.3 *

High density L1210-DFMO^r cells were diluted in fresh medium containing serum, supplemented with opioid agonists at the concentration of 10μM and harvested after 16 h. Results are means ± S.D. of three separate determinations. ** $P < 0.01$ and * $P < 0.05$, significantly different from control.

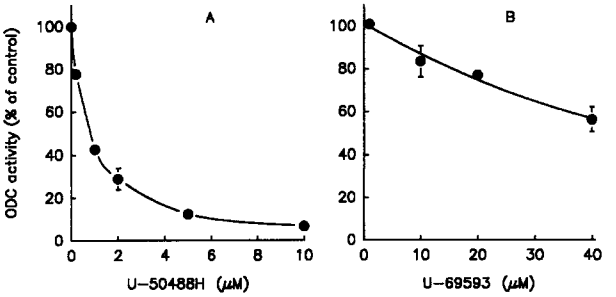

Fig. 1. Effect of increasing concentration of U-50488H (panel A) and U-69593 (panel B) on ODC induction in L1210-DFMO^r cells. High density cells were diluted in fresh medium containing serum and treated with different concentrations of the κ agonists. Cells were harvested 16 h after seeding. Results are means ± S.D. of three separate determinations.

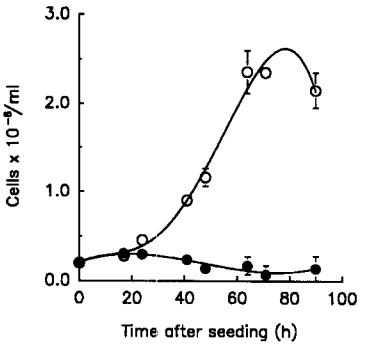

Fig. 2. Effect of U-50488H on growth curve of L1210-DFMO^r cells. Cells were diluted and incubated in the absence (○) or presence (●) of 10 μM U-50488H. Results represent means ± S.D. of three separate flasks.

less than 50% of control already after 2 h of treatment ($P < 0.01$) and then continued to decline.

The negative effect of U-50488H on the induction of ODC activity was accompanied by a marked decrease of immunoreactive ODC protein (data not shown), and by a reduction of ODC mRNA, as detected by combined reverse transcriptase/PCR analysis (Fig. 4). In fact the level of ODC mRNA, very low in quiescent cells, increased after dilution of the cells, and U-50488H treatment reduced this increase. Quantitation of ODC mRNA content by kinetic PCR followed by capillary electrophoresis (see Materials and Methods) showed that the level of the messenger was about 40% and 20% of control after 8 h and 16 h with U-50488H, respectively. In addition, a 2.7-fold increase in the ODC decay following cycloheximide was observed in cells treated for 16 h with U-50488H (Fig. 5A). Since U-50488H provoked a rapid decrease of preinduced ODC activity (Fig. 3B), ODC turnover was analyzed even after a short treatment with the drug. Fig. 5B shows that under these conditions U-50488H affected ODC turnover slightly.

Finally, the effect of U-50488H on the induction of ODC activity in wild type L1210 cells was also examined: ODC activity was 6.7 U/mg protein 16 h after cell

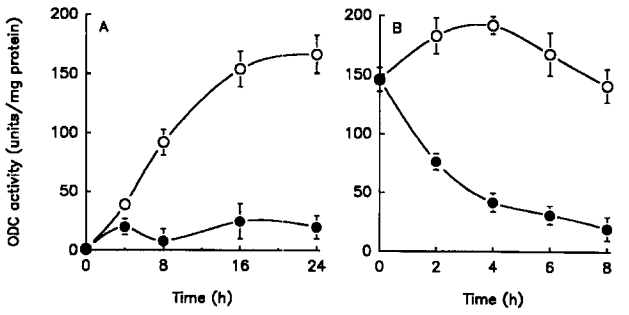

Fig. 3. Effect of U-50488H on the time-course of ODC activity in L1210-DFMO^r cells. Panel A: cells were diluted and immediately treated with 10 μM U-50488H. Panel B: cells were diluted and, after 16 h, treated with 10 μM U-50488H. (○), control cells; (●), U-50488H treated cells. Results are means ± S.D. of three separate determinations.

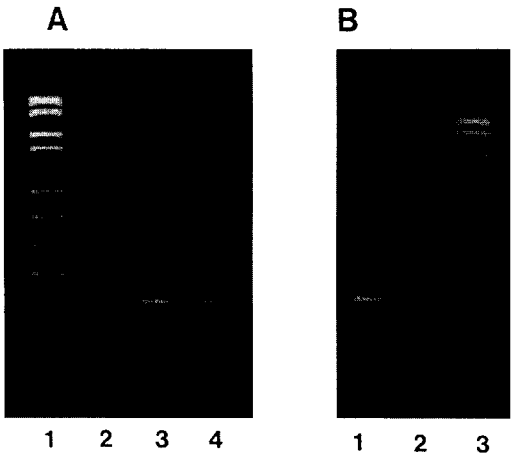

Fig. 4. Effect of U-50488H on the expression of ODC mRNA. L1210-DFMO^r cells were harvested and analyzed for ODC mRNA by reverse transcriptase/PCR. PCR products (224 bp) were visualized by ethidium bromide fluorescence after separation on gel agarose electrophoresis. Panel A: lane 1, molecular size markers (*BglI* and *HinfI* pBR328 DNA fragments of 2176, 1766, 1230, 1033, 653, 517, 453, 394, 298, 234, 220 and 154 bp); lane 2, high density quiescent cells; lane 3, control cell (8 h after dilution); lane 4, cells treated with 10 μM U-50488H for 8 h. Panel B: lane 1, control cells (16 h after dilution); lane 2, cells treated with 10 μM U-50488H for 16 h; lane 3, molecular size markers.

dilution (control) and 3.6 and 1.4 U/mg protein in cells treated with 10 μM and 50 μM U-50488H, respectively.

3.2. Effects of opioid antagonists and stereospecificity of U-50488H

Since U-50488H is considered a selective κ opioid agonist, some experiments were carried out to verify if the effects on ODC induction could be mediated by classical

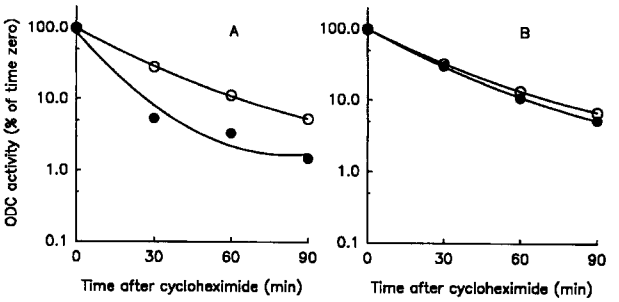

Fig. 5. Effect of U-50488H on the rate of ODC decay. Panel A: cells were diluted and half of them immediately treated with 10 μM U-50488H. After 16 h, 0.2 mM cycloheximide was added to all the cells and samples were removed at 30-min intervals. Average ODC activity of U-50488H treated cells at time zero was 9.5% of control. Panel B: cells were diluted and, after 16 h, half of them were treated with 10 μM U-50488H; 1 h later, 0.2 mM cycloheximide was added to all the cells and samples were removed at 30 min intervals. Average ODC activity of U-50488H treated cells at time zero was 70.4% of control. (○), control cells; (●), U-50448H treated cells. The results represent means of two separate experiments. U-50488H treatment affected ODC decay significantly in each experimental condition ($P < 0.05$ vs. control by paired *t*-test).

Table 2
Effect of some opioid antagonists on the U-50488H inhibition of ODC induction

Antagonist	Concn. (μM)	ODC activity (% of control) U-50488H concn.(μM)		
		0	1	2
none	–	100.0 ± 6.9	63.2 ± 3.0	36.3 ± 2.2
naloxone	5	89.3 ± 2.7	64.1 ± 0.5	36.9 ± 4.3
	20	103.5 ± 14	66.7 ± 5.4	36.6 ± 4.8
Mr1452	1	93.1 ± 12	67.0 ± 1.0	n.d.
	10	74.3 ± 5.9	57.6 ± 6.0	26.1 ± 3.4
nBNI	2	92.4 ± 1.1	63.2 ± 6.3	30.7 ± 4.7
	10	71.9 ± 5.1	52.9 ± 5.1	23.2 ± 3.0

L1210-DFMO^r cells were diluted and treated with U-50488H in the presence or absence of opioid antagonists. Cells were harvested 8 h after seeding. Results are mean ± S.D. of three determinations; n.d., not determined. Mr1452 and nBNI exerted a significant effect on ODC induction ($P < 0.01$ by two-way ANOVA).

opioid receptors. Table 2 shows that neither naloxone, nor the selective κ antagonists Mr1452 and nBNI were able to block the ODC inhibiting effect of U-50488H in L1210-DFMO^r cells. Actually the κ selective antagonists showed a negative effect, although less marked than U-50488H. Pretreatment with the alkylating, irreversible opioid antagonist β-CNA (0.5 μM) was also completely ineffective in blocking the U-50488H action on ODC and had no effect per se. Furthermore the separated enantiomers (−) and (+) of U-50488H (U-53444E and U-53445E) were similarly effective and inhibited ODC induction to a degree comparable to the racemic compound (U-50488H) (results not shown). The (−)-*cis*-(1*S*,2*R*)-U50488 stereoisomer, exhibiting preference for σ receptors, and carbetapentane, another σ agonist, were also effective, although to a lesser extent compared to U-50488H (Table 3). Other pharmacological agents tested did not affect ODC activity (tetrodotoxin, carbachol), or, in the case of atropine, reduce it only slightly.

Table 3
Effect of various pharmacological agents on the induction of ODC activity

Agent	concn. (μM)	ODC activity (% of control)
U-50488H	1	51.4 ± 10.1 **
	10	11.4 ± 2.1 **
(−)-*cis*-(1*S*,2*R*)-U-50488	1	94.1 ± 1.2
	10	31.9 ± 3.3 **
Carbetapentane	1	85.6 ± 5.8 *
	10	42.9 ± 3.6 **
Tetrodotoxin	2	106.2 ± 4.8
	10	108.9 ± 2.8
Carbachol	10	99.4 ± 8.2
	100	97.3 ± 2.1
Atropine	10	84.2 ± 1.4 *

L1210-DFMO^r cells were diluted and treated with the indicated agents. Cells were harvested 16 h after seeding. Results are means ± S.D. of three determinations. ** $P < 0.01$ and * $P < 0.05$, significantly different from control. In addition, the effects of carbetapentane or (−)-*cis*-(1*S*,2*R*)-U50488 were significantly different from the effect of U-50488H ($P < 0.01$ by two-way ANOVA).

4. Discussion

This research shows that U-50488H, a selective κ-agonist widely used in the pharmacological and biochemical research [33], can inhibit the expression of ODC activity and the growth of L1210-DFMO^r cells. The inhibiting effect of U-50488H on ODC activity appeared to result from both a reduction in the level of ODC mRNA and an acceleration of ODC turnover. Concentrations similar to those used in the present research or even higher concentrations of U-50488H have been employed in in vitro systems with intact cells or sinaptosomes to show significant effects [2,3,34–36]. Interestingly, U-50488H at 10 μM was found to increase thymidine incorporation into DNA by 60% in rat spinal cord-dorsal root ganglion co-cultures [3]. In another study with fetal rat brain cell aggregates, U-50488H (1 μM) either increased or decreased DNA synthesis according to the stage of brain development [2].

U-69593, another κ opioid selective agonist [2], was slightly effective in the present study; however the effects of U-50488H on ODC induction do not appear to involve κ or other classical opioid receptors, lacking both enantiospecificity and antagonist sensitivity. Actually, the κ-antagonists nBNI and Mr1452 were themselves inhibitory. Other effects of U-50488H not mediated by classical opioid receptors have been reported [11,37–40]. Interestingly, U-50488H inhibited nicotine-induced Ca^{2+}-uptake into cultured adrenal medullary cells with a IC_{50} of 1 μM and none of various opioid antagonists examined was able to reverse this effect [11]. Furthermore the κ antagonist Mr2266 also produced a marked inhibition of Ca^{2+} uptake.

Although U-50488H shows a marked preference for κ-receptors, it also can bind the naloxone-insensitive σ sites, the (−) enantiomer exhibiting a slightly higher affinity than the (+) compound [41]. Even if σ receptors have been identified in lymphoid cells [42], the U-50488H effect on ODC does not seem to be mediated by σ sites. In fact the (−)-*cis*-U-50488 isomer, exhibiting a high affinity for σ receptors [41], proved to be less potent than the trans isomers. Furthermore haloperidol, which has been reported to antagonize σ effects of U-50488H [43] and metaphit, which can act as an irreversible inhibitor of σ receptors [44], could not block the U-50488H effect on ODC (data not shown). These findings however do not rule out the possibility that σ-sites may be involved in the control of ODC expression, as suggested by the efficacy of carbetapentane, another σ ligand [12].

It is possible to speculate that in lymphoid cells U-50488H may bind to a receptor whose natural ligand is unknown. Recently an atypical opioid receptor has been

cloned and characterized [44]: it did not bind any of the prototypical endogenous opioids (β-endorphin, enkephalin and dynorphin) but only the non selective opioid ligand etorphine at relatively high concentration. As an alternative speculation, U-50488H may bind to some post-receptor site. For instance, Peryasamy et al. [45] hypothesized that U-50488H inhibited carbachol-stimulated phosphoinositide turnover in rat hippocampal slices through a direct interaction with a G protein. Stimulation of some muscarinic receptors has been shown to have mitogenic effects [46,47]; however, in our conditions, atropine was hardly effective in reducing ODC induction and carbachol neither affected ODC induction nor prevented the U-50488H inhibiting effect (not shown). Another possible naloxone insensitive site of action of U-50488H, i.e. sodium channels [38–40], seems to be excluded by the lack of effect of tetrodotoxin, which on the contrary reproduced most of the cardiovascular actions of U-50488H [39].

Finally, wild type L1210 cells were also sensitive to U-50488H treatment, indicating that this phenomenon is not limited to a particular, ODC overproducing cell line. However higher concentrations of U-50488H were required to achieve a marked inhibition of ODC induction. Although the reason for this difference is unknown, it may be noted that L1210-DFMO^r cells contain higher levels of polyamines [24], which may modulate signal transduction pathways [48] and regulate ODC itself [15]. Quite recently, we have found that micromolar concentrations of U-50488H inhibit DNA synthesis in both L1210 cell lines and in thymocytes (thymic lymphocytes). Furthermore, U-50488H also inhibited mitogen-elicited ODC induction and promote apoptosis of thymocytes. None of these effects were antagonized by naloxone (manuscript in preparation).

In conclusion this research shows that U-50488H and some related κ-specific ligands can inhibit ODC induction in L1210 cells through a site distinct from the classical, stereospecific opioid receptors, and linked to inhibition of lymphoid cell proliferation.

Acknowledgements

The careful secretarial work of Ms. Angela Zarri and the excellent technical assistant of Ms. Alessandra Mantovani and Ms. Lucia Carletti are acknowledged. This research was supported by grants from M.U.R.S.T. (quota 40% and 60%), Italy.

References

[1] Barg, J., Belcheva, M.M. and Coscia, C.J. (1992) J. Neurochem. 59, 1145–1152.
[2] Barg, J., Belcheva, M.M., Rowinski, J. and Coscia, C.J. (1993) J. Neurochem. 60, 1505–1511.
[3] Barg, J., Nah, S.-Y., Levy, R., Saya, D. and Vogel. Z. (1993) Brain Res. 629, 109–114.
[4] Maneckjee, R., Biswas, R. and Vanderhaas, B.K. (1990) Cancer Res. 50, 2334–2338.
[5] Maneckjee, R. and Minna, J.D. (1990) Proc. Natl. Acad. Sci. USA 87, 3294–3298.
[6] Bryant, H.U. and Holaday, J.D. (1993) Handbook of experimental pharmacology, 104/I, 361–392.
[7] Van Den Bergh, P., Rozing, J. and Nagelkerken, L. (1991) Immunology 72, 537–543.
[8] Taub, D.D., Eisenstein, T.K., Geller, E.B., Adler, M.W. and Rogers, T.J. (1991) Proc. Natl. Acad. Sci. USA 88, 360–364.
[9] Lawrence, D.M.P. and Bidlack, J.M. (1993) J. Pharmacol. Exp. Ther. 266, 1678–1683.
[10] Moises, H.C. and Walkner, J.M. (1985) Eur. J. Pharmacol. 108, 85–98.
[11] Bunn, S.J. and Dunkley, P.R. (1991) Biochem. Pharmacol. 41, 715–722.
[12] Quirion, R., Bowen, W.D., Itzhak, Y., Junien, J.L., Musacchio, J.M., Rothman, R.B., Su, T.-P., Tam, S.W. and Taylor, D.P. (1992) Trends Pharmacol. Sci. 13, 85–86.
[13] Chen, L., Gu, Y. and Huang, L.-Y. (1995) J. Physiol. 482, 575–581.
[14] Pegg, A.E. (1988) Cancer Res. 48, 759–774.
[15] Heby, O. and Persson, L. (1990) Trends Biochem. Sci. 15, 153–158.
[16] Abrahamsen, M.S. and Morris, D.R. (1991) in Perspectives on Cell Regulation: Bacteria to Cancer (Inouye, M., Campisi, J., Cunningham, D.D. and Riley, M., eds.), pp. 107–119, John Wiley and Sons, New York.
[17] Auvinen, M., Paasinen, A., Andersson, L.C. and Hölttä, E. (1992) Nature 360, 355–358.
[18] Butler, S.R. and Schanberg, S.M. (1975) Biochem. Pharmacol. 24, 1915–1918.
[19] Slotkin, T.A., Lau, C. and Bartolome, M. (1976) J. Pharmacol. Exp. Ther. 199, 141–148.
[20] Bartolome, J.V., Bartolome, M.B., Daltner, L.A., Evans, C.J., Kuhn, C.M. and Schanberg, S.M. (1986) Life Sci. 38, 2355–2362.
[21] Bartolome, J.V., Bartolome, M.B., Harris, E.B. and Schanberg, S.M. (1987) J. Pharmacol. Exp. Ther. 240, 895–899.
[22] Benalal, D. and Bachrach, U. (1985) Biochem. J. 227, 389–395.
[23] Baudry, M., Shahi, K. and Gall, C. (1988) Brain Res. 464, 313–318.
[24] Persson, L., Holm, I. and Heby, O. (1988) J. Biol. Chem. 263, 3528–3533.
[25] Flamigni, F., Campana, G., Carboni, L., Rossoni, C. and Spampinato S. (1994) Biochim. Biophys. Acta 1201, 101–105.
[26] Flamigni, F., Stefanelli C., Guarnieri, C. and Caldarera, C.M. (1986) Biochim. Biophys. Acta 882, 377–383.
[27] Seely, J.E. and Pegg, A.E. (1983) J. Biol. Chem. 258, 2496–2500.
[28] Bradford, M. (1976) Anal. Biochem. 72, 248–254.
[29] Chomczynsky, P. and Sacchi N. (1987) Anal. Biochem. 162, 156–159.
[30] Halmekytö, M., Hyttinen, J.-M., Sinervirta, R., Utriainen, M., Myöhänen, A., Vopio, H.-M., Wahlfors, J., Syrjänen, S., Syrjänen, K., Alhonen, L. and Jänne, J. (1991) J. Biol. Chem. 266, 19746–19751.
[31] Sugiyama, S., Hattori, K., Hayakawa, M. and Ozawa, T. (1991) Biochem. Biophys. Res. Commun. 180, 894–899.
[32] Flamigni, F., Campana, G., Carboni, L., Guarnieri, C. and Spampinato, S. (1994) Biochem. J. 299, 515–519.
[33] Vonvoigtlander, P.F., Lahti, R.A. and Ludens, J.H. (1983) J. Pharmacol. Exp. Ther. 224, 7–12.
[34] Fletcher, G.H. and Chiappinelli, V.A. (1993) Neuroscience 53, 239–250.
[35] Olson, K.G. and Welch, S.P. (1991) Life Sci. 48, 575–581.
[36] Ventura, C., Guarnieri, C., Stefanelli, C., Cirielli, C., Lakatta, E.G. and Capogrossi, M.C. (1991) Biochem. Biophys. Res. Commun. 179, 972–978.

[37] Hayes, A.G., Birch, P.P., Cavicchini, E. (1988) J. Pharm. Pharmacol. 40, 718–720.
[38] Alzheimer, C. and Bruggencate, G.T. (1990) J. Pharmacol. Exp. Ther. 255, 900–905.
[39] Pugsley, M.K., Penz, W.P., Walker, M.J.A. and Wong, T.M. (1992) Br. J. Pharmacol. 105, 521–526.
[40] Pugsley, M.K., Saint, D.A. and Walkner, M.J.A. (1994) Eur. J. Pharmacol. 261, 303–309.
[41] De Costa, B.R., Bowen, W.D., Hellewell, S.B., George, C., Rothman, R.B., Reid, A.A., Walkner, J.M., Jacobson, A.E. and Rice, K.C. (1989) J. Med. Chem. 32, 1996–2002.
[42] Wolfe, S.A.Jr., Kulsakdinum, C., Battaglia, G., Jaffe, J.H. and De Soluza, E.B. (1988) J. Pharmacol. Exp. Ther. 247, 1114–1119.
[43] Young, G.A., Marquiz, K.L., Paquette, N.C., Gussio, R.P. and Khazan, N. (1989) Neuropharmacology 28, 881–884.
[44] Bluth, L.S., Rice, K.C., Jacobson, A.E. and Bowen, W.D. (1989) Eur. J. Pharmacol. 161, 273–277.
[45] Molleran, C., Parmentier, M., Mailleux, P., Butour, J.-L., Moisand, C., Chalon, P., Caput, D., Vassart, G. and Mennier, J.-C. (1994) FEBS Lett. 341, 33–38.
[46] Peryasamy, S. and Hoss, W. (1991) Eur. J. Pharmacol. 207, 101–109.
[47] Gutkind, J.S., Novotny, E.A., Braun, M.R. and Robbins, K.C. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 4703–4707.
[48] Thomas, T.J., Gunnia, U.B., Seibold, J.R. and Thomas, T. (1995) Biochem. J. 311, 175–182.